United States Patent Cahoon, et al.

6,593,514 July 15, 2003

Method for the production of calendic acid, a fatty acid containing delta-8,10,12 conjugated double bonds and related fatty acids having a modification at the delta-9 position

### **Abstract**

The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with modification of the delta-9 position of fatty acids, in particular, formation of conjugated double bonds are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles. The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with formation of a trans delta-12 double bond also are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles.

Inventors: Cahoon; Edgar Benjamin (Wilmington, DE); Hitz; William Dean

(Wilmington, DE); Ripp; Kevin G. (Wilmington, DE)

Assignee: E. I. du Pont de Nemours and Company (Wilmington, DE)

Appl. No.: 638937

Filed: August 15, 2000

Current U.S. Class:

Current C.S. Class.

**800/281**; 800/298; 435/69.1; 435/419; 536/23.6 A01H 005/00; C12N 015/82; C07H 021/04

Intern'l Class:

A0111 003/00, C1211 013/02, C0711 021/04

Field of Search: 800/281,298 435/69.1,419 536/23.6

References Cited [Referenced By]				
U.S. Patent Documents				
<u>4945050</u>	Jul., 1990	Sanford et al.		
<u>5107065</u>	Apr., 1992	Shewmaker et al.		
<u>5231020</u>	Jul., 1993	Jorgensen et al.		
<u>5428072</u>	Jun., 1995	Cook et al.		
<u>5519451</u>	May., 1996	Clatanoff et al.		
<u>5554646</u>	Sep., 1996	Cook et al.		
<u>5851572</u>	Dec., 1998	Cook et al.		
Foreign Patent Documents				
0 242 236	Aug., 1996	EP.		
94/11516	May., 1994	WO.		

sequences required for activity of the cauliflower mosaic virus 35S Promoter. Mark D. Adams et al., Science, vol. 252:1651-1656, 1991, Complementary DNA Sequencing: Expressed Sequence Tags and Human Genome Project. M. D. Chisholm et al., Can. Journ. of Biochem., vol. 42:1033-1040, 1964, Biosynthesis of Mustard Oil Glucosides.

R. C. Thiel et al., J. Anim. Sci., vol. 77(suppl):47, 1998, Effects of CLA supplementation on quality and sensory characteristics of pork.

R. C. Wiegand et al., J. Anim. Sci., vol. 77(suppl):47, 1999, Effects of CLA supplementation on pork quality characteristics in crossbred growing-finishing barrows.

Linda Gritz et al., Gene, vol. 25: 179-188, 1983, Plasmid-encoded hygromycin B resistance: the sequence of hygromycin B phosphotransferase gene and its expression in Escherichia coli and Saccharomyces cerevisiae.

Mats Hamberg et al., Biochem. & Biophys. Res. Comm., vol. 188(3):1992, Metabolism of 6,9,12-Octadecatrienoic acid in the red alga lithothamnion corallioides: Mechanism of formation of a conjugated tetraene fatty acid. Edgar B. Cahoon et al., J. Biol. Chem., vol. Manuscript Moo9188200, No. in press, 2000, pp. 1-27, Formation of conjugated delta 8, delta 10 double bonds by delta 12-oleic acid desaturase related enzymes: Biosynthetic origin of calendic acid.

Kathrin Fritsche et al., FEBS Lett., vol. 462:249-253, 1999, Isolation and characterization of a calendic acid producing (8,11)-linoleoyl desaturase.

Primary Examiner: McElwain; Elizabeth F.

### Parent Case Text

This application claims priority benefit of U.S. Provisional Application No. 60/149,050 filed Aug. 16, 1999, now abandoned.

### Claims

- 1. A chimeric gene comprising an isolated *nucleic acid* fragment encoding a plant fatty acid modifying enzyme associated with conjugated double bond formation comprising a delta-9 position of fatty acids having an amino acid *identity* of at least 72.5% based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2 or 4 wherein said fragment or a functionally equivalent subfragment thereof or a complement thereof is operably linked to suitable regulatory sequences.
- 2. The chimeric gene of claim 1 wherein the *nucleic acid* fragment is isolated from

Polypeptides having peroxidase activity and nucleic acids encoding same

### **Abstract**

The present invention relates to isolated polypeptides having peroxidase activity and isolated nucleic acid sequences encoding the polypeptides. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides.

Inventors: Yaver; Debbie (Davis, CA); McArdle; Barbara (Davis, CA)

Assignee: Novozymes Biotech, Inc. (Davis, CA)

Appl. No.: 885329

Filed: June 19, 2001

Current U.S. Class: 435/192; 435/6; 435/320.1; 435/325; 435/252.3;

536/23.1; 536/23.2

Intern'l Class: C12N 009/08; C12N 015/00; C12N 005/00; C12Q

001/68; C07H 021/04

Field of Search: 435/192,6,252.3,320.1 536/23.2,23.1

### References Cited [Referenced By]

### Other References

Mester et al., 1998, Journal of Biochemistry 273: 15412-15417.

Primary Examiner: Monshipouri; M.

Attorney, Agent or Firm: Stames; Robert L.

### Parent Case Text

### CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. application Ser. No. 09/596,824 filed Jun. 19, 2000 now U.S. Pat. No. 6,372,464 issued Apr. 16, 2002, which application is fully incorporated herein by reference.

### Claims

- 1. An isolated *nucleic acid* sequence encoding a polypeptide having peroxidase activity, selected from the group consisting of:
- (a) a *nucleic acid* sequence encoding a polypeptide having an amino acid sequence which has at least 75% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6, or at least 85% *identity* with amino acids 22 to 385 of SEQ ID NO:4;
- (b) a *nucleic acid* sequence encoding a polypeptide having an amino acid sequence which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5, or at least 85% homology with nucleotides 2008 to 3462 of SEQ ID NO:3;
- (c) a *nucleic acid* sequence which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) the cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii); and
- (d) a fragment of (a), (b), or (c), which encodes a polypeptide having peroxidase activity.
- 2. The *nucleic acid* sequence of claim 1, which encodes a polypeptide having an amino acid sequence which has at least 75% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.
- 3. The *nucleic acid* sequence of claim 2, which encodes a polypeptide having an amino acid sequence which has at least 80% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.
- 4. The *nucleic acid* sequence of claim 3, which encodes a polypeptide of having an amino acid sequence which has at least 85% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.
- 5. The *nucleic acid* sequence of claim 4, which encodes a polypeptide having an amino acid sequence which has at least 90% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.
- 6. The *nucleic acid* sequence of claim 5, which encodes a polypeptide having an amino acid sequence which has at least 95% *identity* with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

- 7. The *nucleic acid* sequence of claim 1, which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.
- 8. The *nucleic acid* sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, or a fragment thereof having peroxidase activity.
- 9. The *nucleic acid* sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO6.
- 10. The *nucleic acid* sequence of claim 1, which encodes a polypeptide which consists of amino acids 22 to 370 of SEQ ID NO:2, amino acids 22 to 365 of SEQ ID NO:4, or amino acids 19 to 362 of SEQ ID NO:6.
- 11. The *nucleic acid* sequence of claim 1, which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
- 12. The *nucleic acid* sequence of claim 11, which has at least 80% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
- 13. The *nucleic acid* sequence of claim 12, which has at least 85% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
- 14. The *nucleic acid* sequence of claim 13, which has at least 90% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
- 15. The *nucleic acid* sequence of claim 14, which has at least 95% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
- 16. The *nucleic acid* sequence of claim 1, which has the *nucleic acid* sequence of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:1.
- 17. The *nucleic acid* sequence of claim 1, which has the *nucleic acid* sequence of nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5.
- 18. The *nucleic acid* sequence of claim 1, which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) me cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2845 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii).
- 19. The *nucleic acid* sequence of claim 1, which is contained in plasmid pBM37-7 which is contained in E. coli NRRL B-30280, plasmid pBM38-1 which is contained in E. coli NRRL B-30281, or plasmid pBM39-1 which is contained in E. coli NRRL B-30282.

Plant polyphenol oxidase homologs

### **Abstract**

This invention relates to an isolated nucleic acid fragment encoding a polyphenol oxidase enzyme. The invention also relates to the construction of a chimeric gene encoding all or a portion of the polyphenol oxidase enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the polyphenol oxidase enzyme in a transformed host cell.

Inventors:

Cahoon: Rebecca E. (Wilmington, DE); Falco; Saverio Carl (Arden, DE);

Kinney; Anthony J. (Wilmington, DE); Miao; Guo-Hua (Hockessin, DE)

Assignee:

E. I. du Pont de Nemours and Company (Wilmington, DE)

Appl. No.:

889463

Filed:

July 16, 2001

PCT Filed:

February 8, 2000

PCT NO:

PCT/US00/03176

PCT PUB.NO.: WO00/47726

PCT PUB. Date: August 17, 2000 **Current U.S. Class:** 

435/190; 435/183; 435/252.3; 435/320.1; 435/71.1;

536/23.2; 536/23.1

Intern'l Class:

C12N 009/04; C12N 001/20; C12N 015/00; C12P

021/04; C07H 021/04

Field of Search:

435/190,183,252.3,320.1,71.1 536/23.2,23.1

# References Cited [Referenced By]

	<b>U.S.</b> 1	Patent Documents	
<u>5786193</u>	Jul., 1998	Greene et al.	435/193.
•	Foreig	n Patent Documents	
93/02195	Feb., 1993	WO.	
93/15599	Aug., 1993	WO.	
97/29193	Aug., 1997	WO.	
98/53080	Nov., 1998	WO.	

Other References

Michelle D. Hunt, et. al., Plant Molecular Biology, vol. 21:59-68, 1993, cDNA Cloning and Expression of Potato Polyphenol Oxidase.

Sally M. Newman, et. al., Plant Molecular Biology, vol. 21:1035-1051, 1993, Organisation of the Tomato Polyphenol Oxidase Gene Family.

National Center for Biotechnology Information General Identifier No. 1172584, Oct. 1, 1996, Boss, P.K., et al., an Apple Polyphenol Oxidase cDNA is Up-Regulated in Wounded Tissues.

Paul K. Boss, et. al., Plant Molecular Biology, vol. 27:429-433, 1995, an Apple Polyphenol Oxidase cDNA is Up-Regulated in Wounded Tissues.

National Center for Biotechnology Information General Identifier No. 1785613, Jan. 18, 1997, Virador, V.M., et. al., Molecular Cloning and c-DNA Sequence of Grenache (Vitis Vinifera) Leaf Polyphenol Oxidase.

National Center for Biotechnology Information General Identifier No. 418754, Jul. 21, 2000, Cary, J.W., et. al., Cloning and Characterization of cDNAS Coding for Vicia Faba Polyphenol Oxidase.

Jeffrey W. Cary, et. al., Plant Molecular Biology, vol. 20:245-253, 1992, Cloning and Characterization of cDNAS Coding for Vicia Faba Polyphenol Oxidase.

National Center for Biotechnology Information General Identifier No. 1172586, Dec. 15, 1998, Cary, J.W., et. al., Cloning and Characterization of cDNAS Coding for Vicia Faba Polyphenol Oxidase.

William H. Flurkey, Plant Phys., VOL 91:481-483, 1989, Polypeptide Composition and Amino-Terminal Sequence of Broad Bean Polyphenoloxidase.

National Center for Biotechnology Information General Identifier No. 451937, Jun. 4, 1999, Haruta, M., et. al., Immunological and Molecular Comparison of Polyphenol Oxidase in Rosaceae Fruit Trees.

Miyoshi Haruta, et. al., Phytochemistry, vol. 50:1021-1025, 1999, Immunological and Molecular Comparison of Polyphenol Oxidase in Rosaceae Fruit Trees.

National Center for Biotechnology Information General Identifier No. 2737882, Jan. 1, 1998, Bucheli, C.S., et. al., Purification of Polyphenol Oxidase and Isolation of a Full Length cDNA from Sugarcane, a C4 Grass.

Primary Examiner:	Achutamurthy;	Ponnathapu
Assistant Examiner	Pak: Yong	

# Parent Case Text

This application claims the benefit of U.S. Provisional Application No. 60/119,590, filed Feb. 10, 1999.

aim	

- 1. An isolated polynucleotide comprising:
- (a) a nucleotide sequence encoding a polypeptide having polyphenol oxidase B activity, wherein the polypeptide has an amino acid sequence of at least 80% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:20, or
- (b) a complement of the nucleotide sequence of (a), wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary.
- 2. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide has at least 85% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:20.
- 3. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide has at least 90% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:20.
- 4. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide has at least 95% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:20.
- 5. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide comprises SEQ ID NO:20.
- 6. The polynucleotide of claim 1 wherein the nucleotide sequence comprises SEQ ID NO:19.
- 7. A vector comprising the polynucleotide of claim 1.
- 8. A recombinant DNA construct comprising the polynucleotide of claim 1 operably linked to at least one regulatory sequence.
- 9. A method for transforming a cell, comprising transforming a cell with the polynucleotide of claim 1.
- 10. A cell comprising the recombinant DNA construct of claim 8.
- 11. A method for production of a polypeptide having polyphenol oxidase B activity comprising the steps of cultivating the cell of claim 10 under conditions that allow for the synthesis of the polypeptide and isolating the polypeptide from the cultivated cells, from the culture medium, or from both the cultivated cells and the culture medium.

Human complement C3-degrading protein from Streptococcus pneumoniae

### Abstract

The present invention relates to the identification and use of a family of human complement C3degrading proteinases expressed by S. pneumoniae. The proteinase has a molecular weight of about 24 kD to about 34 kD as determined on a 10% SDS polyacrylamide gel. A preferred proteinase of this invention includes the amino acid sequence of SEQ ID NO:2.

Inventors:

Hostetter; Margaret K. (New Haven, CT); Dunny; Gary (St. Paul, MN);

Nandiwada; Lakshmi S. (Mendota Heights, MN)

Assignee:

Regents of the University of Minnesota (Minneapolis, MN)

Appl. No.:

403422

Filed:

October 19, 1999

PCT Filed:

April 24, 1998

PCT NO:

PCT/US98/08281

PCT PUB.NO.: WO98/48022

PCT PUB. Date: October 29, 1998

Current U.S. Class:

**424/190.1**; 424/184.1; 424/185.1; 424/234.1; 424/237.1;

424/244.1; 424/69.1; 424/320.1; 536/23.1; 536/23.7;

530/324; 530/350; 530/380

Intern'l Class:

A61K 039/02

Field of Search:

530/300,350,380,324

424/184.1,185.1,190.1,234.1,237.1,244.1,94.1 514/1

536/23.1,23.2 435/69.1,320.1

### References Cited [Referenced By]

	U.S. 1	Patent Documents	
<u>4902506</u>	Feb., 1990	Anderson et al.	
<u>5360897</u>	Nov., 1994	Anderson et al.	•
5476929	Dec., 1995	Briles et al.	
<u>5510264</u>	Apr., 1996	Van Alstyne et al.	
<u>5614382</u>	Mar., 1997	Metcalf.	
	Foreign	Patent Documents	
0 622 081	Nov., 1994	EP.	•
0 687 688	Dec., 1995	EP.	

Assistant Examiner: Zeman; Robert A.

Attorney, Agent or Firm: Mueting Raasch & Gebhardt

### **Government Interests**

### STATEMENT OF GOVERNMENT SUPPORT

The invention was made with the support of National Institutes of Health grant number R01-AI24162. The U.S. government may have certain rights to the invention.

### Parent Case Text

This patent application claims benefit of Provisional application Ser. No. 60/044,316 filed Apr. 24, 1997.

### Claims

- 1. An isolated and purified protein comprising at least an 80% sequence identity of SEQ ID NO:2 and that binds human complement protein C3.
- 2. The protein of claim 1 wherein the protein is isolated and purified from S. pneumoniae.
- 3. The protein of claim 1, wherein the protein is a recombinant protein.
- 4. The protein of claim 1 having a molecular weight as determined on a 10% polyacrylamide gel of between about 24 kDa to about 34 kDa.
- 5. The protein of claim 4, wherein the protein is isolated and purified from S. pneumoniae.
- 6. The protein of claim 4 wherein the protein is a recombinant protein.
- 7. The protein of claim 4 wherein the protein degrades human complement protein C3.
- 8. A peptide comprising at least 15 sequential amino acids from the protein of claim 1.
- 9. A composition comprising the peptide of claim 8.
- 10. The composition of claim 9, further comprising an adjuvant.

- 11. A composition comprising the protein of claim 1.
- 12. The composition of claim 11 further comprising an adjuvant.
- 13. An isolated and purified protein comprising SEQ ID NO:2.
- 14. A composition comprising the protein of claim 13.
- 15. The composition of claim 14 further comprising an adjuvant.
- 16. A peptide comprising at least 15 sequential amino acids set forth in SEQ ID NO:2, wherein said peptide binds human complement protein C3.
- 17. A composition comprising the peptide of claim 16.
- 18. The composition of claim 17 further comprising an adjuvant.
- 19. A protein comprising amino acids 1-50 of SEQ ID NO:2.
- 20. A composition comprising the protein of claim 19.
- 21. The composition of claim 20 further comprising an adjuvant.
- 22. An isolated and purified protein that binds human complement protein C3 and wherein nucleic acid encoding the protein hybridizes to SEQ ID NO:1 under hybridization conditions of 6.times.SSC, 5.times.Denhardt, 0.5% SDS, and 100 .mu.g/ml fragmented and denatured salmon sperm DNA hybridized overnight at 65.degree. C. and washed in 2.times.SSC, 0.1% SDS one time at room temperature for about 10 minutes followed by one time at, 65.degree. C. for about 15 minutes followed by at least one wash in 0.2.times.SSC, 0.1% SDS at room temperature for at least 3-5 minutes.
- 23. A composition comprising the protein of claim 22.
- 24. The composition of claim 23 further comprising an adjuvant.
- 25. An immunogenic composition comprising an isolated and purified polypeptide comprising SEQ ID NO:2.
- 26. The composition of claim 25 wherein the polypeptide is isolated and purified from S. pneumoniae.
- 27. The composition of claim 25 further comprising at least one other immune stimulating peptide, polypeptide or protein from S. pneumoniae.
- 28. An isolated nucleic acid fragment that hybridizes to SEQ ID NO:1 under hybridization

conditions of 6.times.SSC, 5.times.Denhardt, 0.5% SDS, and 100 .mu.g/ml fragmented and denatured salmon sperm DNA hybridized overnight at 65.degree. C. and washed in 2.times.SSC, 0.1% SDS one time at room temperature for about 10 minutes followed by one time at, 65.degree. C. for about 15 minutes followed by at least one wash in 0.2.times.SSC, 0.1% SDS at room temperature for at least 3-5 minutes, wherein said isolated nucleic acid fragment encodes a polypeptide that binds human complement protein C3.

- 29. The nucleic acid of claim 28 isolated from an S. pneumoniae genome.
- 30. The nucleic acid of claim 28 wherein the polypeptide degrades human complement C3.
- 31. The nucleic acid fragment of claim 28 wherein the nucleic acid fragment encodes a polypeptide that does not degrade human complement C3.
- 32. The nucleic acid of claim 28 in a nucleic acid vector.
- 33. The nucleic acid of claim 32 wherein the vector is an expression vector.
- 34. An isolated host cell comprising the nucleic acid vector of claim 32.
- 35. The cell of claim 34 wherein the cell is a bacterium or a eukaryotic cell.
- 36. An isolated host cell comprising the isolated nucleic acid of claim 28.
- 37. A method of expressing a polypeptide that binds to human complement protein C3, the method comprising culturing a recombinant host cell transformed with an isolated nucleic acid fragment of claim 28 under conditions suitable for expression of a polypeptide and recovering the polypeptide so expressed.
- 39. A method for producing an immune response to S. pneumoniae in an animal comprising the steps of:

administering a composition comprising a polypeptide comprising SEQ ID NO:2 to a mammal; and

obtaining an immune response to the polypeptide in said animal.

- 40. The method of claim 39 wherein the immune response is a B cell response.
- 41. The method of claim 39 wherein the immune response is a T cell response.
- 42. The method of claim 39 wherein the composition further comprises at least one other protein

from S. pneumoniae.

- 43. A bacteria comprising a nucleic acid comprising an insertional mutation, wherein said mucleic acid encodes a protein of claim 1.
- 44. The bacteria of claim 43 wherein the insertional mutation comprises an insertional duplication mutation.
- 45. An isolated and purified of about 24 kDa to about 34 kDa from Streptococcus pneumoniae that binds to human complement C3.
- 46. An isolated nucleic acid fragment comprising the nucleic acid sequence of SEQ ID NO:1.
- 47. The isolated nucleic acid fragment of claim 46 wherein the nucleic acid fragment encodes a protein that binds human complement C3.
- 48. An isolated RNA fragment transcribed from a double-stranded DNA sequence comprising SEQ ID NO:1.
- 49. An isolated nucleic acid fragment that encodes a polypeptide having at least an 80% sequence identity with SEQ ID NO:2 and binds human complement protein C3.
- 50. The isolated nucleic acid fragment of claim 49, said isolated nucleic acid fragment encoding a polypeptide comprising SEQ ID NO:2.

Elongase genes and uses thereof

### **Abstract**

The subject invention relates to the identification of several genes involved in the elongation of polyunsaturated acids (i.e., "elongases") and to uses thereof. At least two of these genes are also involved in the elongation of monounsaturated fatty acids. In particular, elongase is utilized in the conversion of gamma linolenic acid (GLA) to dihomogamma linolenic acid (DGLA) and in the conversion of AA to adrenic acid (ADA), or eicosapentaenoic acid (EPA) to .omega.3-docosapentaenoic acid (DPA). DGLA may be utilized in the production of polyunsaturated fatty acids, such as arachidonic acid (AA), docosahexaenoic acid (DHA), EPA, adrenic acid, .omega.6-docosapentaenoic acid or .omega.3-docosapentaenoic acid which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

Inventors: Mukerji; Pradip (Gahanna, OH); Leonard; Amanda Eun-Yeong (Gahanna, OH);

Huang; Yung-Sheng (Upper Arlington, OH); Pereira; Suzette L. (Westerville, OH)

Assignee: Abbott Laboratories (Abbott Park, IL)

Appl. No.: 903456

Filed: July 11, 2001

Current U.S. 435/193; 435/252.33; 435/320.1; 435/348; 435/419; 435/254.11; 435/254.21;

Class: 435/254.22; 435/254.3; 435/254.4; 435/254.5; 435/254.6; 435/252.31;

435/328; 435/254.23; 536/23.2

Intern'l Class: C12N 009/10; C12N 005/10; C12N 001/20; C12N 001/15; C12N 015/00;

C12N 005/06; C12N 005/16; C07H 021/04; 254.6; 252.31; 328; 254.23

Field of Search: 435/193,252.33,320.1,252.3,348,419,254.11,254.21,254.22,254.3,254.4,254.5

#### References Cited [Referenced By] U.S. Patent Documents Horrobin et al. May., 1987 4666701 Horrobin et al. Jul., 1988 4758592 Stewart et al. 4826877 May., 1989 Houck et al. Jul., 1990 4943674 Comai et al. Apr., 1992 5106739 Horrobin et al. May., 1992 <u>5116871</u> Martineau et al. Dec., 1992 5175095

<u>5188958</u>	Feb., 1993	Moloney et al.
<u>5196198</u>	Mar., 1993	Shaw et al.
<u>5420034</u>	May., 1995	Kridl et al.
<u>5443974</u>	Aug., 1995	Hitz et al.
<u>5463174</u>	Oct., 1995	Molony et al.
5484724	Jan., 1996	El-Sherbeini et al.
<u>5552306</u>	Sep., 1996	Thomas et al.
5589379	Dec., 1996	Kridl et al.
<u>5700671</u>	Dec., 1997	Prieto et al.
<u>5750176</u>	May., 1998	Prieto et al.
	Foreign	Patent Documents
88/07577	Oct., 1988	WO.
93/11245	Jun., 1993	WO.
94/11516	May., 1994	WO.
95/24494	Sep., 1995	WO.
96/13591	May., 1996	WO.
98/39448	Sep., 1998	WO.

### Other References

Lassner, et al., The Plant Cell 8:281-292 (1996).

Oh, et al., The Journal of Biological Chemistry, 272 (28):17376-17384 (1997).

Schnieke, et al., Science, 278:2130-2133 (1997).

Horrobin, et al., Am. J. Clin. Nutr., vol. 57 (Suppl.) 732S-737S.

Brenner, et al., Adv. Exp. Med. Biol., 83:85-101 (1976).

Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444-2448 (1988).

Knutzon, et al., J. Biol. Chem. 273:29360-29366 (1998).

Hoveland, et al., Gene 38:57-64 (1989).

Ausubel, et al., Short Protocols in Molecular Biology, Ch. 13:3-5 (1992).

Gietz, et al., Mol. Cell, Biol, 5:255-269 (1995).

Hoffman, et al., Gene, 57:267 (1987).

Altschul, et al., Nuc. Acids Res., 25:3389-3402 (1997).

Primary Examiner: Prouty; Rebecca E.
Assistant Examiner: Swope; Sheridan
Attorney, Agent or Firm: Becker; Cheryl L.

### Parent Case Text

The subject application is a Continuation-In-Part of U.S. patent application Ser. No. 09/624,670 filed on Jul. 24, 2000, which is a Continuation-In-Part of pending U.S. patent application Ser. No. 09/379,095 filed on Aug. 23, 1999, which is a Continuation-In-Part of U.S. patent application Ser. No. 09/145,828 filed on Sep. 2, 1998 now U.S. Pat. No. 6,403,349 issued Jun. 11, 2002, all of which are herein incorporated in their entirety by reference.

### Claims

- 1. An isolated nucleic acid sequence comprising or complementary to a nucleic acid sequence encoding a polypeptide having elongase activity, wherein the amino acid sequence of said polypeptide has at least 80% amino acid sequence identity to SEQ ID NO:7.
- 2. The isolated nucleic acid sequence of claim 1 wherein said sequence comprises SEQ ID NO:7.
- 3. The isolated nucleic acid sequence of claims 1 or 2 wherein said sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate.
- 4. The isolated nucleic acid sequence of claim 1 wherein said sequence is derived from the genus Thraustochytrium.
- 5. The isolated nucleic acid sequence of claim 4 wherein said sequence is derived from Thraustochytrium aureum.
- 6. A method of producing an elongase enzyme comprising the steps of:
- a) isolating a nucleotide sequence comprising SEQ ID NO:7 (FIG. 72);
- b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.
- 7. The method of claim 6 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.
- 8. The method of claim 7 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.
- 9. The method of claim 7 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

- 10. The method of claim 9 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.
- 11. The method of claim 10 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.
- 12. The method of claim 11 wherein said yeast cell is Saccharomyces cerevisiae.
- 13. A vector comprising: a) a nucleotide sequence comprising SEQ ID NO:7 (FIG. 72) operably linked to b) a promoter.
- 14. A host cell comprising said vector of claim 13.
- 15. The host cell of claim 14 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.
- 16. The host cell of claim 15 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.
- 17. The host cell of claim 15 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.
- 18. The host cell of claim 17 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.
- 19. The host cell of claim 18 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.
- 20. The host cell of claim 19 wherein said yeast cell is Saccharomyces cerevisiae.
- 21. A plant cell comprising said vector of claim 13, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell.
- 22. The plant cell of claim 21 wherein said polyunsaturated fatty acid is selected from the group consisting of dihom-.gamma.-linolenic acid (DGLA), 20:4n-3, adrenic acid (ADA) and .omega.3-docosapentaenoic acid.

Plant metabolism genes

### **Abstract**

This invention relates to an isolated nucleic acid fragment encoding a GTP cyclohydrolase II/3,4-dihydroxy-2-butanone-4-phosphate synthase protein. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of the protein, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the protein in a transformed host cell.

Inventors: Allen; Stephen M. (Wilmington, DE); Kinney; Anthony J. (Wilmington, DE); Rafalski; J. Antoni (Wilmington, DE); Orozco, Jr.; Emil M. (West Grove, PA); Miao; Guo-Hua (Johnston, IA); Famodu; Omolayo O. (Newark, DE); Lee; Jian-Ming (West Caldwell, NJ); Lohman; Karin N. (Newark, DE); Rendina; Alan R. (Wilmington, DE); Sakai; Hajime (Wilmington, DE); Weng; Zude (Des Plaines, IL); Caimi; Perry G. (Kennet Square, PA); Fang; Yiwen (Los Angeles, CA); Shen; Jennie Bih-Jien (Wilmington, DE); Zoughi; Ilham L. (Wilmington, DE); Anderson; Shawn L. (West Grove, PA); Shi; Jinrui (Johnston, IA); Lu; Guihua (Urbandale, IA); Helentjaris; Timothy G. (Ankeny, IA); Li; Chun Ping (Johnston, IA)

Assignee: E.I. du Pont de Nemours and Company (Wilmington, DE); Pioneer Hi-Bred

International (Des Moines, IA)

Appl. No.: 614912

Filed: July 12, 2000

Current U.S. Class: 800/278; 435/6; 435/69.1; 435/183; 435/410; 435/419;

435/252.3; 435/320.1; 530/350; 530/370; 536/23.2; 536/23.6; 536/24.1; 800/295

Intern'l Class: A01H 003/00; C07H 021/04; C07K 014/415; C12N

005/14; C12N 009/00

Field of Search: 435/6,69.1,183,410,419,252.3,320.1 530/350,370

536/23.2,23.6,24.1 800/278,295

# U.S. Patent Documents Nov., 1997 Meyerowitz et al. 5824868 Oct., 1998 Meyerowitz et al.

Foreign Patent Documents

95/35383 Dec., 1995 WO.

### Other References

M. Humbelin et al., J. of Ind. Microbiol & Biotech., vol. 22:1-7, 1999, GTP cyclohydrolase II and 3,4-dihydroxy-2-butanone 4-phosphate synthase are rate-limiting enzymes in riboflavin synthesis of an industrial Bacillus subtilis strain used for riboflavin production.

A. Bacher et al., Methods in Enzymol. vol. 280:382-389, Biosynthesis of Riboflavin: GTP Cyclohydrolase II, Deaminase, and Reductase.

National Center for Biotechnology Information General Identifier No. 1346113, May 30, 2000, Kobayashi, M. et al., Isolation of cDNAs encoding GTP cyclohydrolase II from Arabidopsis thaliana.

Masahiko Kobayashi et al., Gene, vol. 160:303-304, 1995, Isolation of cDNAs encoding GTP cyclohydrolase II from Arabidopsis thaliana.

National Center for Biotechnology Information General Identifier No, 2462925, Oct. 2, 1997, Herz, S.W.

National Center For Bio Technology Information General Identifier No. 10188005, Sep. 16, 2000, Herz et al.

National Center For Biotechnology Information General Identifier No. 19552807, Nakagawa, S. Oct. 1, 2002.

Bork. Genome Research, vol. 10, 2000, p. 398-400.\*

Lazar et al. Molecular and Cellular Biology, Mar. 1988, vol. 8, No. 3, p. 1247-1252.\* Burgess et al. The Journal of Cell Biology, 1990, vol. 111, p. 2129-2138.\*

Broun et al. Science, Nov. 13, 1998, vol. 282, p. 131-133.

Primary Examiner: Bui; Phuong T.

### Parent Case Text

This application claims the benefit of U.S. Provisional Applications No. 60/143,401 filed Jul. 12, 1999; No. 60/143,412, filed Jul. 12, 1999; No. 60/146,650, filed Jul. 30, 1999; No. 60/170,906 filed Dec. 15, 1999; No. 60/172,959 filed Dec. 21, 1999; No. 60/172,946 filed Dec. 21, 1999.

### Claims

- 1. An isolated polynucleotide comprising:
- (a) a nucleotide sequence encoding a polypeptide having GTP cyclohydrolase II/3,4-dihydroxy-2-butanone-4-phosphate synthase activity, wherein the amino acid sequence of the polypeptide

and the amino acid sequence of SEQ ID NO:66 have at least 80% sequence identity, or

- (b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.
- 2. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:66 have at least 85% sequence identity.
- 3. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:66 have at least 90% sequence identity.
- 4. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:66 have at least 95% sequence identity.
- 5. The polynucleotide of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:66.
- 6. The polynucleotide of claim 1, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:65.
- 7. A vector comprising the polynucleotide of claim 1.
- 8. A recombinant DNA construct comprising the polynucleotide of claim 1 operably linked to at least one regulatory sequence.
- 9. A method for transforming a cell comprising transforming a cell with the polynucleotide of claim 1.
- 10. A cell comprising the recombinant DNA construct of claim 8.
- 11. A method for producing a plant comprising transforming a plant cell with the polynucleotide of claim 1 and regenerating a plant from the transformed plant cell.
- 12. A plant comprising the recombinant DNA construct of claim 1.
- 13. A seed comprising the recombinant DNA construct of claim 1.

Phosphatidylcholine biosynthetic enzymes

### **Abstract**

This invention relates to an isolated nucleic acid fragment encoding phosphatidylethanolamine N-methyltransferase biosynthetic enzyme. The invention also relates to the construction of a chimeric gene encoding all or a portion of the phosphatidylethanolamine N-methyltransferase biosynthetic enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of phosphatidylethanolamine N-methyltransferase biosynthetic enzyme in a transformed host cell.

Inventors: Famodu; Omolayo O. (Newark, DE); Kinney; Anthony J. (Wilmington,

DE): Rafalski; J. Antoni (Wilmington, DE)

Assignee: E. I. du Pont de Nemours and Company (Wilmington, DE)

Appl. No.: 668262

Filed: September 22, 2000

Current U.S. Class: 800/281; 435/6; 435/69.1; 435/183; 435/410;

435/419; 435/252.3; 435/320.1; 530/350; 530/370;

536/23.2; 536/23.6; 536/24.1; 536/24.3; 536/24.33;

800/278; 800/295

Intern'l Class: A01H 003/00; C07H 021/04; C07K 014/415; C12N

005/14; C12N 009/00

Field of Search: 435/6,69.1,183,410,419,252.3,320.1 530/350,370

536/23.2,23.6,24.1,24.3,24.33 800/278,295,281

### References Cited [Referenced By]

### Other References

Michael J. Homann et al., Journal of Bacteriology, vol. 169(7):3276-3280, Jul. 1987, Coordinate Regulation of Phospholipid Biosynthesis by Serine in Saccharomyces cerevisiae.

Xiaoying Lin et al., Nature, vol. 402:761-768, Dec. 16, 1999, Sequence and Analysis of Chromosome 2 of the plant Arabidopsis thaliana.

Patricia McGraw et al., Genetics, vol. 122:317-330, Jun. 1989, Mutations in the Saccharomyces cerevisiae opi3 Gene: Effects on Phospholipid Methylation, Growth and Cross-Pathway Regulation of Inositiol Synthesis.

National Center for Biotechnology Information General Identifier No. 3786005, Apr. 5, 2000, Lin, X. et al., Sequence and Analysis of Chromosome 2 of the

Bork. Genome Research, vol. 10, 2000, p. 398-400.

Primary Examiner: Bui; Phuong T.

### Parent Case Text

This application claims the benefit of U.S. Provisional Application No. 60/155,626, filed Sep. 23, 1999.

### Claims

- 1. An isolated polynucleotide comprising:
- (a) a nucleotide sequence encoding a polypeptide having phosphatidylethanolamine N-methyltransferase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 80% sequence *identity* based on the Clustal alignment method, or
- (b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.
- 2. The *polynucleotide* of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 85% sequence *identity* based on the Clustal alignment method.
- 3. The *polynucleotide* of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 90% sequence *identity* based on the Clustal alignment method.
- 4. The *polynucleotide* of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 95% sequence *identity* based on the Clustal alignment method.
- 5. The *polynucleotide* of claim 1 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:20.
- 6. The *polynucleotide* of claim 1 wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:19.

- 7. A vector comprising the *polynucleotide* of claim 1.
- 8. A recombinant DNA construct comprising the *polynucleotide* of claim 1 operably linked to a regulatory sequence.
- 9. A method for transforming a cell comprising transforming a cell with the *polynucleotide* of claim 1.
- 10. A cell comprising the recombinant DNA construct of claim 8.
- 11. A method for producing a plant comprising transforming a plant cell with the *polynucleotide* of claim 1 and regenerating a plant from the transformed plant cell.
- 12. A plant comprising the recombinant DNA construct of claim 8.
- 13. A seed comprising the recombinant DNA construct of claim 8.

Nucleic acid molecules from plants encoding enzymes which participate in starch synthesis

### **Abstract**

Nucleic acid molecules are described which encode enzymes which participate in starch synthesis in plants. These enzymes are a new isoform of starch synthase. There are furthermore described vectors for generating transgenic plant cells and plants which synthesize a modified starch. There are furthermore described methods for the generation of these transgenic plant cells and plants, and methods for producing modified starches.

Inventors: Frohberg; Claus (Berlin, DE)

Assignee: Aventis CropScience GmbH (Frankfurt, DE)

Appl. No.: 638524

Filed:

August 11, 2000

Foreign Application Priority Data

Aug 11, 1999[DE] 199 37 348

Current U.S. Class: 800/284; 800/278; 800/286; 800/320.1; 435/69.1;

435/101; 435/320.1; 435/419; 435/468; 536/23.6

Intern'l Class: C12N 015/29; C12N 015/82; C12N 005/04; A01H

005/00; C12P 019/04

Field of Search: 536/23.6 435/69.1,468,320.1,419,101

800/278,284,320.1,286

# References Cited [Referenced By]

U.S. Patent Documents	
Oct., 2000	Kossmann et al.
Foreign Patent Documents	
Jul., 1997	CA.
Nov., 1997	CA.
Nov., 1997	DE.
Jun., 1998	DE.
Jun., 1997	EP.
May., 1996	WO.
Jul., 1997	WO.
	Oct., 2000  Foreign Pate Jul., 1997 Nov., 1997 Nov., 1997 Jun., 1998 Jun., 1997 May., 1996

WO 98/27212

Jun., 1998

WO.

WO 99/24575

May., 1999

WO.

### Other References

Nakatani et al. Jpn. J. Crop Sci. 61(3): 463-468, 1992.\*

Kossmann et al. Progress Biotechnol. 10:271-278, 1995.\*

Sasaki, T. Accession No. C99345, 1996.\*

Database Accession No. C99345 published Oct. 16, 1998, also referred to as XP 002163786.

Database Accession No. AI94842 published Aug. 23, 1999, also referred to as XP 002163787.

Cao, Heping et al, "Identification of the Soluble Starch Synthase Activities of Maize Endosperm", Plant Physiology, May 1999, vol. 120, pp. 205-215, No. 1.

Primary Examiner: Fox; David T.

Attorney, Agent or Firm: Frommer Lawrence & Haug LLP

### Claims

### I claim:

- 1. An isolated *nucleic acid* molecule encoding a protein with the bioactivity of a starch synthase selected from the group consisting of
- (a) nucleic acid molecules which encode a protein with the amino acid sequence indicated under SEQ ID No. 2;
- (b) nucleic acid molecules which encompass the nucleotide sequence shown under SEQ ID No. 1 or a complementary sequence thereof;
- (c) nucleic acid molecules which encompass the coding region of the nucleotide sequence of the cDNA present in plasmid IR 65/87 (deposit number DSM 12970) or a complementary sequence thereof;
- (d) nucleic acid molecules whose nucleotide sequence deviates from the sequence of the nucleic acid molecules mentioned under (a), (b) or (c) owing to the degeneracy of the genetic code;
- (e) nucleic acid molecules which have over 85% sequence identity with SEQ ID NO:1; and
- (f) nucleic acid molecules which constitute allelic variants of the nucleic acid molecules

Plant glucose-6-phosphate translocator

### Abstract

This invention relates to an isolated nucleic acid fragment encoding a glucose-6-phosphate/phosphate translocator. The invention also relates to the construction of a chimeric gene encoding all or a portion of the glucose-6-phosphate/phosphate translocator, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the glucose-6-phosphate/phosphate translocator in a transformed host cell.

Inventors: Allen; Stephen M. (Wilmington, DE); Rafalski; J. Antoni (Wilmington,

DE)

Assignee: E. I. du Pont de Nemours and Company (Wilmington, DE)

Appl. No.: 436521

Filed: November 9, 1999

Current U.S. Class: 800/278; 435/6; 435/69.1; 435/71.1; 435/183;

435/410; 435/419; 435/418; 435/252.3; 435/320.1;

530/350; 530/370; 536/23.1; 536/23.2; 536/23.6;

536/24.1; 536/24.3; 536/24.5

Intern'l Class: A01H 003/00; C07H

A01H 003/00; C07H 021/04; C07K 014/415; C12N

005/14; C12N 009/00

Field of Search:

435/6,69.1,71.1,183,410,419,418,252.3,320.1 530/370,350 536/23.1,23.2,23.6,24.1,24.3,24.5

### References Cited [Referenced By]

**U.S. Patent Documents** 

5945509

Aug., 1999

Heinemann et al.

**Foreign Patent Documents** 

WO 95/16913

Jun., 1995

WO.

### Other References

Lazar et al. Molecular and Cellular Biology, vol. 8, No. 3, p. 1247-1252, Mar. 1988.\*

Burgess et al. The Journal of Cell Biology, vol. 111 p. 2129-2138, 1990.\* Brown et al. Science, vol. 282, p. 131-133, Nov. 1998.\*

Bork. Genome Research vol. 10, p. 398-400, 2000.\* Kammerer, B. et al. (1998) The Plant Cell 10:105-117. Denyer et al. (1996) Plant Physiol. 112:779-785. Thorbjornsen et al. (1996) Plant J. 10:243-250. NCBI General Identifier No. 2997591. NCBI General Identifier No. 2997589.

Primary Examiner: Bui; Phuong T.

### Parent Case Text

This application claims priority benefit to U.S. Provisional Application No. 60/107,910 filed Nov. 10, 1998, now abandoned.

### Claims

- 1. An isolated polynucleotide comprising:
- (a) a nucleotide sequence encoding a polypeptide having glucose-6-phosphate/phosphate translocator activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 86% sequence *identity* based on the Clustal alignment method, or
- (b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.
- 2. The *polynucleotide* of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 90% sequence *identity* based on the Clustal alignment method.
- 3. The *polynucleotide* of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 95% sequence *identity* based on the Clustal alignment method.
- 4. The *polynucleotide* of claim 1, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:3.
- 5. The *polynucleotide* of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:4.

Method for activating only the vascular endothelial growth factor receptor-3 and uses thereof

### Abstract

A method for activating only the vascular endothelial growth factor receptor-3 has been created. The method comprises administration of a composition comprising a polypeptide to an animal wherein the composition has the ability to stimulate one or more lymphatic vessel endothelial cells to proliferate, differentiate, migrate, or survive. Methods are also provided to selectively activate a VEGF receptor-3, to screen for neoplastic disease characterized by an increase in lymph vessel endothelial cells, and to identify lymph vessel endothelial cells.

Inventors: Achen; Marc (Parkville, AU); Stacker; Steven (Parkville, AU)

Assignee: Ludwig Institute for Cancer Research (New York, NY)

Appl. No.: 847524

Filed: May 3, 2001

**Current U.S. Class:** 

**424/85.1**; 530/351

Intern'l Class:

A61K 038/19

Field of Search:

424/85.1 530/351 435/7.1,325

## References Cited [Referenced By]

### Other References

Achen et al. VEGF-D is a ligand for the VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). 1998. Proc. Natl. Acad. Sci. USA, 95:548-553.

Primary Examiner: Spector; Lorraine Assistant Examiner: Jiang; Dong

Attorney, Agent or Firm: Crowell & Moring LLP

### Claims

### What is claimed is:

1. A method for stimulating proliferation and/or maintaining of only lymph vessel endothelial cells, in a mammal in need of such treatment, comprising:

administering to said cells an effective amount of a composition comprising a polypeptide having at least a 90% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof which has the ability to only stimulate lymphatic vessel endothelial cells to proliferate, differentiate, migrate or survive.

- 2. The method of claim 1, wherein the polypeptide has at least a 95% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof.
- 3. The method of claim 2, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof.
- 4. The method of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6.
- 5. A method for activating only a VEGF receptor-3, comprising:

administering to a cell bearing said receptor an effective amount of a composition comprising a polypeptide having at least 90% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof which has the ability only to activate a VEGF receptor 3.

- 6. The method of claim 5, wherein the polypeptide has a 95% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof.
- 7. The method of claim 6, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof.
- 8. The method of claim 7, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6.
- 9. The method of claim 5, wherein the method is carried out in vivo.
- 10. The method of claim 5, wherein the method is carried out in vitro.